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A method for separating, extracting and purifying Poly-β-hydroxyalkanoates (PHAs) directly from bacterial fermentation broth

Substitute Specification - Marked-up Copy

(As Previously Amended under PCT Article 34)

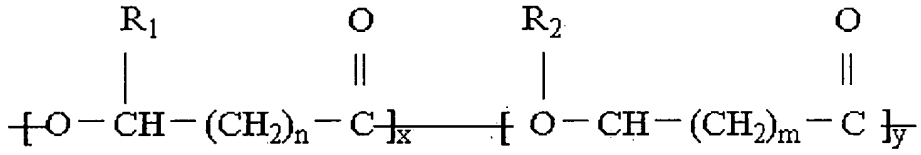
Technical field

This invention relates to post treatment of biological engineering, particularly to extraction and separation of bacterial fermentation products, ~~product,~~ or more particularly to extraction and separation of polyhydroxyalkanoates in cells.

Background of invention

Poly-β-hydroxyalkanoates (PHAs) are biological polyesters accumulated in cells by special microorganisms under special growth conditions.

~~General formula:~~ The general formula of poly-β-hydroxyalkanoates is:



In which, n and m are integers from 1 to 4, and typically ~~1~4 integer, usually it is~~ 1, that is for example 3-hydroxyalkanoates (3-HAS); R_1 and R_2 are straight chain or branched chain C_{1-12} alkyl groups which are substituted or non-substituted; X and Y are not 0 simultaneously, and determine the content of the component in copolymer. The average molecular weight of PHAs is generally 1-4 million Da.

The physical property of PHAs is similar to that of polypropylene. As its biodegradability, biocompatibility, ~~piezoelectricity~~ piezoelectric properties and optical activity are characteristics not possessed by common petrochemical resins, it has wide application ~~prospect~~ prospects in industry, agriculture, medicine, sanitation, food, electronics, etc.

Large scale industrialized production of PHAs has not been realized internationally. The principal reason is because the cost is much higher than that of

petrochemical resin. The cost of PHAs includes mainly material cost and separation purification cost. The material cost depends on production efficiency of bacteria species and fermentation technology, whilst the separation purification cost depends largely on technology. The current extraction technology includes separation of cells from fermentation liquid with high speed centrifuge and purification of PHAs in separated wet bacterial body with organic solvent extraction, chemical reagent or surfactant + enzyme. These methods suffer from ~~have the defect of~~ high cost or serious pollution, and are difficult to be industrialized. ~~One~~ A one step extraction separation method for extracting polyhydroxyalkanoates directly from fermentation liquid containing cells is disclosed in Chinese patent application CN1328160A, but it must use large quantities ~~quantity~~ of sodium hypochlorite and has the defect of poor operation environment, serious pollution, cost increased by waste water treatment, and product quality affected by shear degradation of PHAs.

~~The~~ Chinese patent No. CN119067A discloses a method for separating and purifying polyhydroxyalkanoates in bacterial cells from bacterial thallus. The method includes two steps in extraction, which requires separation of bacterial thallus from fermentation liquid. As the bacterial thallus is very small (several microns) and the fermentation liquid has certain viscosity, it is necessary to use high speed centrifuge of separation factor higher than 6000. This greatly increases ~~greatly~~ the investment and is a bottleneck in large scale industrialized production. The method also requires expensive protease to treat the separated product which increases cost.

The extraction method disclosed in Chinese patent CN1171410A requires high speed centrifuge and lyophilization of product separated with centrifuge. These are very difficult in large scale industrialized production. Taking a factory producing 100,000 tons of PHAS/year as example, nearly 3000-4000 tons of fermentation liquid/day will be centrifuged and 600-800 tons will be lyophilized. The industrial cost will be very high. The final washing with organic solvent pollutes the environment and increases product cost.

U.S. Patent No 4,910,145 ~~US patent US4910145A~~ discloses a method for separating and extracting PHAS with enzyme and surfactant. Since the cell wall and membrane are very complicated in composition and cannot be completely decomposed

with one enzyme, it is necessary to use several enzymes or a compound enzyme. As the optimal action conditions as pH, temperature, etc. of different enzymes are different, the technology is very complicated. The method requires heating fermentation liquid to >80°C which consumes enormous energy. The price of enzyme preparation is high, so the separation cost is high. Besides, the purity of product is not high.

The purpose of this invention is to provide an extraction method for PHAs, which can ~~reduce~~ effectively reduce separation and purification cost, reduces pollution, and is suitable for industrialized production.

Invention

This invention provides a method for directly separating and purifying polyhydroxyalkanoates in cells from bacterial fermentation liquid, which comprises: ~~comprisings:~~

(1) pretreating fermentation liquid with a physical method for breaking cell wall;

(2) adjusting the pH ~~value~~ of the pretreated fermentation liquid so that it is alkaline;

(3) adding anionic surfactant and agitating;

(4) separating and extracting coagulated precipitate in reaction liquid;

(5) washing and drying.

The physical method includes mechanical breaking and ultrasonic breaking. The sequence of adjusting pH and adding surfactant is interchangeable. The mechanical breaking includes ball milling or high pressure homogenization.

In step 3, aside from adding anionic surfactant, coagulating agent can be added.

The mechanical breaking method used to break cell wall can be ball milling.

The pH of the pretreated fermentation liquid is adjusted to 8-13. The alkaline substance used in adjusting pH can be solid or an aqueous solution of NaOH, Na_2CO_3, $NaHCO_3$ or ammonia water.

The anionic surfactant can be olefinesulfonate (AOS), a fatty alcohol sulfate, a fatty alcohol polyoxyethylene-ether sulfate (AES), a fatty alcohol-polyoxyethylene ether (AEO), alkylphenol-polyoxyethylene ether, ~~etc., its~~ etc. The quantity of anionic surfactant is 0.5-20% (W/V) of fermentation liquid.

The coagulating agent is sodium polyacrylate, modified starch, polyamine, ~~etc.; its~~ etc. The quantity of the coagulating agent is 0.5-20% (W/V) of the fermentation liquid.

After adding the anionic surfactant and the coagulating agent, the reaction temperature under agitation is 10-70°C and the reaction time 5-60min.

Centrifuge, filter-press, vacuum suction filtration, etc. can be used for separating and extracting coagulated precipitate from the reaction liquid.

The invention is applicable to separation and purification of fermentation liquid of bacteria and its aberrance and gene engineering bacteria containing polyhydroxyalkanoates. Applicable bacteria include Alcaligenes, Pseudomonas, Azotobacter, Rhodospirillum, Methylotrophs, Bacillus, etc.

The invention has no high requirement for dry weight of cells and content of PHAs in fermentation liquid. The invention has the advantage of simple technology, low cost, high yield and greatly reduced pollution, so large scale industrialized production can be realized.

Detailed description of the invention

The following ~~Following~~ examples are used to further describe the invention. These examples should not be construed as limiting ~~constitute any limitation to~~ the scope of claimed invention. ~~claims.~~ Any modifications or changes made by the skilled man in the art benefited ~~benefit~~ from the disclosure of this application should be included within the scope of claims stated in this application.

Example 1

~~Take~~ 1000ml of fermentation liquid of Alcaligenes entrophus mutant 65-7, in which the dry weight of cells ~~is 142g/l;~~ was 142g/l and the content of PHBV ~~is 78.5%; pretreat~~ was 78.5% was pretreated with a ball mill (530r/min, 0.1mm steel ball) for ~~40min; adjust~~ 40 min. The pH ~~value~~ of the resulting solution was adjusted to 12 with 30% NaOH solution. ~~solution; add~~ 13g of sodium ~~laurylsulfate; adjust reaction~~ laurylsulfate was added to the solution and the temperature was adjusted to ~~32°C; react under~~ 32°C while the solution was subjected to agitation for ~~5min; filter~~ 5 min. The

solution was filtered with suction and filter ~~paper; wash~~ paper. The coagulated precipitate was washed with water ~~till~~ until the washing ~~becomes neutral; dry at 70°C to constant weight. Purity~~ liquid became neutral, then dried at 70°C. The purity of the product ~~is~~ was 98.2%. The ~~98.2%, the~~ average molecular weight ~~is~~ was 5.2 x 10^5 Da. The ~~5.2x10^{5}Da, the~~ yield ~~is~~ was 85.2%. ~~the~~ The COD and BOD of waste water from suction filtration after treatment with anaerobic and aerobic bacteria is 800 and 30mg/l respectively, in conformity with state discharge standards. ~~standard~~.

Example 2

~~Take~~ 100ml of fermentation liquid of Alcaligenes entrophus, in which the dry weight of cells ~~is 147g/l ,~~ was 147g/l and the content of PHBV ~~is 75.2%; break cell wall~~ was 75.2% was subject to a process that broke the cell walls with ultrasonic (1500W) for ~~20min; adjust~~ 20 min. The pH ~~value~~ of the resulting solution was adjusted to 8 with 30% NaOH ~~solution; add~~ solution. 0.5g of sodium laurylsulfate and 5g of sodium polyacrylate were added to the solution and the ~~polyacrylate; adjust reaction~~ temperature of the solution was adjusted to ~~70°C; react under~~ 70°C while the solution was subjected to agitation for ~~30min; filter~~ 30 min. The solution was filtered with suction and filter ~~paper; wash~~ paper. The coagulated precipitate was washed with water ~~till~~ until the washing ~~becomes neutral; dry in oven at 70°C to constant weight. Purity~~ liquid became neutral, then dried at 70°C. The purity of the product was 93.2%. The ~~is 93.2%, the~~ average molecular weight ~~is 4.1x10^{5}Da, the yield is 80.3%.~~ was 4.1 x 10^5 Da. The yield was 80.3%.

Example 3

~~Take~~ 50ml of fermentation liquid of Alcaligenes entrophus, in which the dry weight of cells ~~is 102g/l,~~ was 102 g/l and the content of PHB ~~is 60%; pretreat~~ was 60% was pretreated with a ball mill (560r/min, 0.1mm steel ball) for ~~30min; adjust~~ 30 min. The pH ~~value~~ of the solution was adjusted to 13 with $NH_3.H_2O$ ~~solution; add~~ solution. 10g of sodium laurylsulfate and 10g of modified ~~starch; adjust reaction~~ starch were added to the solution and the temperature of the solution was adjusted to ~~10°C; react under~~ 10°C while the solution was subjected to agitation for ~~10min; separate with centrifuge~~ 10 min. The solution was centrifuged (separation factor ~~600); wash~~ 600) and the coagulated

precipitate was washed with water ~~till~~ until the washing ~~becomes neutral; dry in oven at 70°C to constant weight. Purity~~ liquid became neutral, then dried at 70°C. The purity of the product ~~is 98.2%, the~~ was 98.2%. The average molecular weight ~~is $4.4x10^5$Da, the~~ was 4.4×10^5 Da. The yield ~~is~~ was 87%.

Example 4

~~Take~~ 500ml of fermentation liquid of Alcaligenes entrophus mutant 65-7, in which the dry weight of cells ~~is 135g/l,~~ was 135 g/l and the content of PHB ~~is 75.5% and introduce~~ was 75.5% was introduced ~~it~~ into a ~~special~~ vessel. ~~Increase~~ The pressure in the vessel was increased to ~~60MPa, release pressure~~ 60 MPa and rapidly released after ~~10min, collect~~ 10 min and the liquid was collected. This operation was repeated ~~and repeat the operation~~ twice. ~~Adjust~~ The pH ~~value~~ of the solution was adjusted to 10 with 30% NaOH ~~solution; add~~ solution. 9g of sodium lauryl polyoxyethylene ether sodium ~~sulfate; adjust reaction~~ sulfate was added to the solution and the temperature of the solution was adjusted to ~~38°C; react under~~ 38°C while the solution was subjected to agitation for ~~8min; filter~~ 8 min. The solution was filtered with suction and filter ~~paper; wash~~ paper. The coagulated precipitate ~~with water till washing becomes neutral; dry at 70°C to constant weight. Purity~~ was washed with water until the washing liquid became neutral. The purity of the product ~~is96.7%, the~~ was 96.7%. The average molecular weight ~~is $4.2x10^5$Da, the~~ was 4.2×10^5. The yield ~~is~~ was 81.5%.

Example 5

~~Take~~ 100ml of fermentation liquid of Alcaligenes entrophus, in which the dry weight of cells ~~is 154g/l,~~ was 154 g/l and the content of PHBV ~~is 80.5%; break cell wall~~ was 80.5% was subject to a process that broke the cell walls with ultrasonic (2800W, continuous treatment) for ~~40min; adjust~~ 40 min. The pH ~~value~~ of the solution was adjusted to 11 with 30% NaOH ~~solution; add~~ solution 10kg of sodium lauryl sulfate and 0.5kg of sodium ~~polyacrylate; adjust reaction~~ polyacrylate was added to the solution and the temperature to ~~50°C; react under~~ 50°C while the solution was subjected to agitation for ~~60min; filter~~ 60 min. The solution was filtered with a filter ~~press; wash~~ press. The coagulated precipitate was washed with water ~~till~~ until the washing became neutral and

the filtrate was dried ~~becomes neutral; dry~~ in oven at 70° to a constant weight. ~~Purity~~ The purity of the product ~~is 97%, the~~ was 97%. The average molecular weight was 5.3 x 10^5 Da. The ~~is 5.3x10^5Da, the~~ yield ~~is~~ was 84%.

Example 6

~~Take~~ 100ml of fermentation liquid of Pseudomonas, in which dry weight of cells was 86 g/l and the ~~is 86g/l,~~ content of PHBV ~~is 61.5%; pretreat~~ was 61.5% was pretreated with a ball mill (560r/min, 0.1mm steel ball) for ~~50min; adjust~~ 50 min. The pH ~~value~~ of the solution was adjusted to 11 with 30% NaOH ~~solution; add~~ solution. 3g of sodium ~~laurylsulfate; adjust reaction~~ laurylsulfate was added to the solution and the temperature of the solution was adjusted to ~~24°C; react under~~ 24°C while the solution was subjected to agitation for ~~10min; filter~~ 10 min. The solution was filtered with suction and filter ~~paper; wash~~ paper. The coagulated precipitate was washed with water ~~till~~ until the washing ~~becomes neutral; dry~~ became neutral. The filtrate was dried at 70°C to a constant weight. ~~Purity~~ The purity of the product ~~is 94.2%, the~~ was 94.2%. The average molecular weight ~~is 3.2x10^5Da, the~~ was 3.2 x 10^5 Da. The yield ~~is~~ was 71.2%.